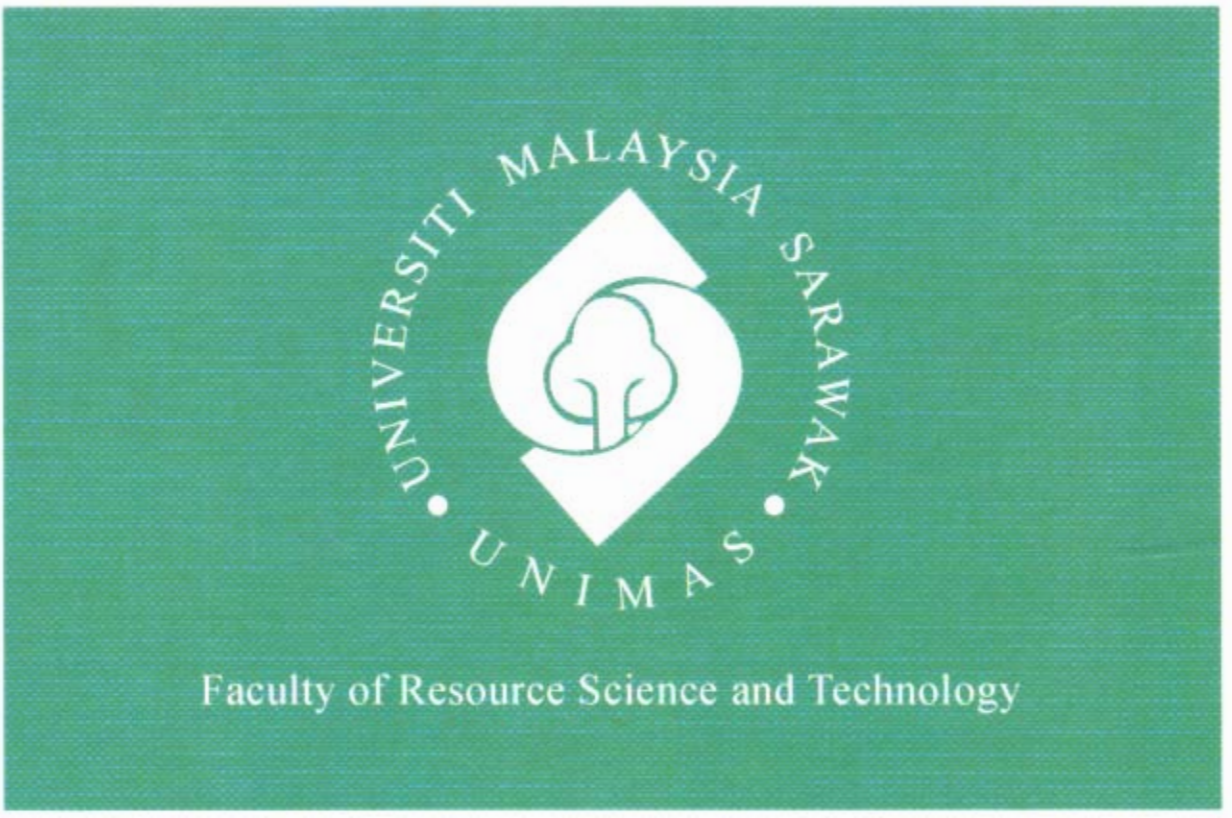

CONTROLLED FED BATCH CULTURE OF *Scenedesmus* FOR PRODUCTION OF LIPIDS

Nur Aqilah Bt Aman

Bachelor of Science with Honours
(Resource Biotechnology)
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Controlled Fed Batch Culture of *Scenedesmus* for Production of Lipids

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DECLARATION

I hereby declare that no portion of this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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LIST OF ABBREAVIATIONS

Ca (NO_3)	Calcium Nitrate
$CaCl.2H_2O$	Calcium Chloride Dihydrate
$Co(NO_3)_2.6H_2O$	Cobalt Nitrate
CO_2	Carbon dioxide
$CuSO_4.5H_2O$	Cupric Sulfate Pentahydrate
DCW	Dry cell weight
dH_2O	Distilled water
DNS	Dinitrosalicyclic acid
$FeCl_3$	Iron (III) Chloride
FSE	Filtered sago effluent
FPW	Filtered pond water
g	gram
g/L	gram per liter
H_3BO_3	Boric acid
K_2HPO_4	Potassium Hydrogen Phosphate
KH_2PO_4	Monopotassium phosphate

L	liter
ml	mili liter
$MgSO_4.7H_2O$	Magnesium Sulphate Hepta Hydrate
$MnSO_4.H_2O$	Manganese Sulfate
NaCl	Sodium Chloride
$NaNO_3$	Sodium Nitrate
Na_2CO_3	Sodium Carbonate
Na_2SiO_3	Sodium Silicate
$(NH_4)_6MO_7O_{24}.4H_2O$	Ammonium Molybdate
OD	Optical desnsity
PGA	Polyglutamic acid
TSS	Total suspended solid
$ZnSO_4.7H_2O$	Zink Sulfate Hepahydrate

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Controlled Fed Batch Culture of *Scenedesmus* for Production of Lipids

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ABSTRACT

Utilization of different medium to culture *Scenedesmus dimorphus* is to determine which medium will produce high biomass and also high lipid yield from *Scenedesmus*. *Scenedesmus* was cultured in Proteose media, filtered sago effluent (FSE) and filtered pond water (FPW). A fed-batch culture with 10% inoculums of *Scenedesmus* was cultured in Proteose media, FSE and FPW using B-Braun fermentor under controlled growth parameter. Culturing of Scenedesmus for each of culture medium in the fermentor is conducted for 20 days. The results showed that culturing of *Scenedesmus* in FSE in the end of cultivation has higher biomass with 508.27 mg/L followed by Proteose medium with 278 mg/L and FPW with 176.40 mg/L. Percentage of lipid yield also shows that growth in FSE produce higher lipid (1.2%) than other culture medium at 1.1 % in Proteose medium and 0.7% in FPW. Hence, cultivation of *Scenedesmus dimorphus* in controlled fed batch culture utilizes FSE medium is the best.

Keywords: *Scenedesmus dimorphus*, Proteose medium, FSE, FPW, biomass and lipid yield.

ABSTRAK

Penggunaan pelbagai jenis media untuk pertumbuhan Scenedesmus dimorphus adalah untuk menentukan media manakah yang akan menghasilkan biomas tertinggi and juga pengeluaran lipid daripada Scenedemus. Scenedesmus dikultur di dalam Proteose media, hampas sago yang ditapis (FSE) dan air kolam yang ditapis (FPW). Kultur fed batch bersama 10% inokulum daripada Scenedesmus dikultur di dalam Proteose media, FSE dan FPW dengan menggunakan fermentor B-Braun di bawah kawalan parameter pertumbuhan. Pengkulturan Scenedesmus bagi setiap kultur media di dalam fermentor B-Braun dijalankan selama 20 hari. Keputusan menunjukkan pengkulturan Scenedesmus di dalam FSE menunjukkan biomas tertinggi dengan 508 mg/L di ikuti oleh media Protease dengan 278 mg/L dan FPW dengan 176.40 mg/L. Peratusan hasil lipid juga menunjukkan pertumbuhan di dalam FSE menghasilkan lipid lebih tinggi (1.2 %) berbanding media kultur lain iaitu 1.1% di dalam Proteose medium dan 0.7% di dalam FPW. Oleh kerana itu, pertumbuhan Scenedesmus dimorphus dalam kultur fed-batch dikendalikan menggunakan media menunjukkan FSE adalah yang terbaik

Kata kunci : *Scenedesmus dimorphus, Proteose media, FSE, FPW, biomas dan hasil lipid.*

1.0 INTRODUCTION

Algae conversion to biofuel technology is now getting broad attention as a new source of renewable energy to alternate the fossil fuels (Hung *et al.*, 2010). Qin (2010) stated that the lipid content in microalgae is 20-40% of algae dry weight. This lipid can be used to produce biodiesel. High lipid productivity of the microalgae subsequently enhances the potential in biodiesel production. Algae biodiesel is highly biodegradable and emission of air toxics and carcinogen that is appears can be reduced compared to petroleum diesel (Lee *et al.*, 2007; Um & Kim, 2008).

Sago palm is a crop that is grown commercially in Malaysia for the sago processing industry. The word 'sago' comes from Javanese which is meaning starch-containing palm pith (Singhal *et al.*, 2008). Sago wastewater from the sago starch processing industry that is discharged can lead to environmental pollution. According to Rashid *et al.* (2010), sago effluent contains huge quantity of organic substance that can cause water pollution. Sago waste produced by sago starch mill is highly contains starch is a major contribution to water pollution around the mill area (Haryanto *et al.*, 1991; Akmar & Kennedy, 2001).

Adeni *et al.* (2010) stated that agro-residues from the starch processing industries are an exploitation that can be further used for environmental conservation and minimize the pollution. Modification of the sago effluent and utilization of the sago waste water can reduce the environmental effect. Previous study proved that elimination of the sago 'hampas' from primary effluent can minimize TSS up to 70% (Bujang *et al.*, 2005; Rashid *et al.*, 2010). Starch and lignocellulosic materials that is contained in the sago 'hampas' offer a suitable choice as substrate in a solid substrate fermentation (Adeni *et al.*, 2010)

According to Becker (1994), *Scenedesmus dimorphus* produce about 16-40% of the dry cell weight. High lipid productivity of *Scenedesmus* offers a promising source of lipid for biodiesel production. Proteose medium (Bristol's medium) is a growth medium specifically for *Scenedesmus*. However, production of this medium is very costly especially in large scale project where *Scenedesmus* culture need to be replicates for research requirement. This problem can be overcome by searching an alternative medium that is cheaper and obtainable. In this study, sago effluent is utilized as a culture medium to find out whether sago effluent is suitable in culturing microalgae such as *Scenedesmus dimorphus*. Sago effluent is rich in starch and suitable medium to use in solid substrate fermentation (Adeni *et al.*, 2010). Moreover, microalgae such as *Scenedesmus dimorphus* can use their starch as their primary storage component (Encarnación *et al.*, 2010). Sago effluent will be filtered first before using it as a culture medium. Pond water is a natural habitat of the cultivation of the microalgae. Filtered pond water also used in this study in cultivation of *Scenedesmus*. Cultivation *Scenedesmus* in Proteose medium is used as a control in this study.

1.1 Research Objectives

From the overview above, the objectives of this study are:

1) To determine best medium of culturing *Scenedesmus* with the highest biomass.
2) To determine medium that will generate high lipid yield.
3) To establish an alternative cheap culture medium in culturing *Scenedesmus*.

2.0 LITERATURE REVIEW

2.1 Sago

2.1.1 Sago Palm

Metroxylon sagu or sago palms is a crop grown commercially especially in Malaysia. Sago starch processing industries is abundantly available in Sarawak. According to Jong (1995), sago palm is known as the "starch crop of the 21st centuries by most of scientist. Adeni *et al.* (2010) also stated that sago has high yield of starch compared to other sources. In Malaysia, sago starch is most abundantly available from pith sago. The pith sago contained sago starch is prepared in a form of small whitish, pinkish or brownish grain that is mostly for foodstuff, stabilizers in pharmaceutical, papers and textiles and plywood (Ali, R.R, Rahman, W.A & Zakaria, N. (n.d).

2.1.2 Sago effluent

Sago waste is one of the agricultural wastes that are abundantly available in Sarawak (Mahamud & Manisah, 2005). Mahamud and Manisah (2005) stated that sago waste actually is an industrial waste after starch is extracted from sago palm processing. Extraction process produces sago effluent and sago waste water that is discharged can lead to environmental pollution. Sago effluent is discharged to the nearby river without any appropriate treatment. Bujang *et. al* (1996) stated that a single sago starch processing mill has been produced approximately 7 tons of sago pith waste daily. According to Environmental Quality Act, 1974 (sewage and industrial effluents regulation, 1979), sago waste water that is discharged from sago mill processing contains high organism materials ('hampas'), chemical oxygen demand (COD), and biological oxygen demand (BOD) that lead to pollution. Therefore, utilization of

industrial waste such as sago effluent for any intention and purpose is considered environmentally sustainable.

2.2 Biodiesel from algae

"Biodiesel is defined as any biomass-derived fuel substitute" (Hung *et al.*, 2010). Algae present a great potential as one of the source in production of the biodiesel. Biodiesel source should have low production costs and large production scale (Singh & Singh, 2010). Biodiesel is produced from transesterification process where chemical reaction between fatty acid (lipid) with alcohol in presence of catalyst to generate alky-ester known as biodiesel. Hung *et al.* (2010) stated performance of biodiesel generated from transesterification process is as well as petroleum diesel. Singh and Singh (2010) stated that algae and seaweed have low production costs and are more obtainable compared than refined oil or recycled oils. According to Janaun and Ellis (2010), biodiesel produced from algae produce high yield non-edible oil production and does not struggle for land with food production. High rates of biomass and oil production of algae due to their simple cellular structure compare to conventional crops makes algae more efficient for biodiesel production (Becker, 1994; Griffiths & Harrison, 2009). Algae can generate large quantities of vegetable oil as a storage product; approximately produce 50% to 60% dry weight as lipid (Sheehan *et al.*, 1998; Griffiths & Harrison, 2009). Matsunaga *et al.* (2009) stated that efficient photosynthetic conversion in algae make a contribution in high lipid content for biodiesel production. Lipid productivity is a crucial factor in choosing algae species for biodiesel production (Griffiths & Harrison, 2009).

2.3 Characteristics of algae

Nowadays, algae are the most promising alternative of biofuel production compared to other sources. Hung *et al.* (2010) stated that the term microalgae are currently being used to cover various oleaginous species. Algae have a simple cellular structure and a large surface to volume body ratio. Due to these feature, microalgae can grow up rapidly and survive in harsh condition (Mata *et al.*, 2010). According to Hung *et al.* (2010), microalgae can be grown in aqueous conditions of both freshwater and saltwater. Rapid reproductive cycles and restricted nutrient necessity are the factor that account algae become the choice in biofuel production (Gordon & Polle, 2007). Utilization of the microalgae as a source in biofuel production can reduce the environment pollution since algae biodiesel contains no sulfur which is non-toxic and highly biodegradable (Um & Kim, 2009).

2.4 Factors influencing algae growth

The main factors affecting the algae growth are light, pH, temperature, agitation and carbon dioxide. However, these growth parameters are dependent on the strain or species of algae.

2.4.1 Effect of light in algae growth

According to Um and Kim (2009), all algae have plastid, the bodies with chlorophyll that carry out photosynthesis. Sufficient light penetration to the algae culture can facilitate algae growth. Light intensity is essential and depends on density of algae culture and the depth of culture. High light intensity and direct sunlight can cause photoinhibition. Photoinhibition is where the light-induced is limited for the photosynthetic capacity of the algae (Encarnación *et al.*, 2010). Overexposed algae

cells to light can lower growth rate and damage. Therefore, Encarnación *et al.* (2010) suggested that $1/10^{th}$ of the amount of light provided that algae only need.

2.4.2 Effects of temperature on algae growth

The most essential limiting factor for culturing algae is temperature. Temperature is a sensitive factor for microalgae growth and metabolic activities in microalgae cells (Xin *et al.*, 2011). Mata *et al.* (2010) stated that algae can tolerate temperature up to 15°C lower than algae optimal temperature but exceeding the optimal temperature by only 2-4°C resulting in the total culture loss. Optimal growth temperature is dependent to the species strain. Encarnación *et al.* (2010) stated that optimal temperature for *Scenedesmus dimorphus* is between 30-35°C.

2.4.3 Effects of pH on algae growth

Changes in the external pH can cause changes in several primary physiology parameters, including internal pH and concentration of other ions in algae culture (Mohan *et al.*, 2011). Commonly, pH range for algae falls between 7 and 9 with the optimum pH range 8.2-8.7 for most algae species (Encarnación *et al.*, 2010). Mohan *et al.* (2011) stated that "pH below 9.0 which is between 8.2 and 8.7 is more favourable for algal growth compared to the neutral and acidic redox conditions".

2.4.4 Effects of agitation on algae growth

Agitation is crucial in culturing algae, especially *Scenedesmus dimorphus* because it will form thick sediment is not agitated constantly. Agitation can increase nutrient availability evenly and receive sufficient and same light distribution to all algae cells in

the culture. According to Encarnación *et al.* (2010), agitation is not only essential to prevent sedimentation of the algae but also make sure that all cells of population are evenly exposed to the light and nutrients and also get better gas exchange between the culture medium and air. However, high speed mechanical mixing and turbulence can damage the algae cells due to shear stress (Mata *et al.*, 2010).

2.4.5 Effects of nutrients and carbon dioxide (CO_2) on algae growth

The level of nutrients can affect algae growth. According to Kong *et al.* (2010), "High levels of nutrients seem to inhibit algae growth in the beginning but provided sustained growth to a high degree". Algae growth not only depends on sufficient supply of essential macronutrient elements and major ions, but also need adequate micronutrient metals (Dragone *et al.*, 2010). Xin *et al.* (2011) stated that limitation in nutrient is one of the most efficient triggers to increase lipid accumulation in single microalgae cell when energy source (light) and carbon source (CO_2 are available). Ying *et al.*, (2009) mentioned that nitrogen source and concentration is crucial in the growth media as it influence the lipid yield from algae. Limitation nitrogen source stimulate mechanism of survival which make cells to stop its division and begins to store energy in the form of lipids (Encarnación *et al.*, 2010). CO_2 is one of the most important nutrients needed for survival which will be converted into biomass (Hung *et al.*, 2010). According to Mohan *et al.* (2011), microalgae utilize carbon source will results in the formation of sugars which further converted to lipids.

2.5 *Scenedesmus dimorphus*

Scenedesmus dimorphus is a green algae, approximately 10µm in size from the class Chlorophyceace. The lipid content of the *Scenedesmus* is 16-40% of the dry cell weight makes this algae is a valuable choice in biodiesel production (Becker, 1994). According to Graham and Wilcox (2000), *Scenedesmus* have fossil records and are implicated in development of certain oil deposits according to their production of decay-resistant cell walls. *Scenedesmus* is categorized as a heavy bacterium and can cause the formation of the thick sediment if not constantly agitated. The optimal growth temperature of the *Scenedesmus* falls between 30-35°C.

2.6 Production of lipid from microalgae

High lipid productivity is an essential characteristic of a species for production of biofuel (Griffiths & Harrison, 2009). Encarnación *et al.* (2010) stated that biodiesel production is depending to the quantity of lipids that algae reach during growth. Microalgae are autotrophic organism and generate their own food during photosynthesis. According to Um and Kim (2009), algae biomass contains proteins, carbohydrates and lipids. Lipids that are present in algae cell is functioning as membrane components, storage products, metabolites and energy supplies (Qin, 2010). Biodiesel production from algae requires conversion lipid to biofuel through transesterification process. Transesterification is a chemical reaction involving triglycerides and alcohol in presence of catalyst to produce alkyl ester that is defined as biodiesel (Sharma & Singh, 2009). Hung *et al.* (2010) analyzed that biodiesel production from microalgae through transesterification process

exhibit similar properties with petroleum diesel fuel and perform as well as petroleum diesel with reducing emission of gaseous pollutants and particulate matter.

2.7 Controlled Fed-batch culture

Fed batch culture is a process acquired removal and addition of culture media in the middle of process at certain of time. Addition of culture media into the cultivation of *Scenedesmus* is known as substrate feeding. According to Saarela *et al.* (2003), due to the major benefits of fed batch process fermentation that combine both batch and continuous process, this type of fermentation widely applied in industrial. In fed batch process, at first the process started as batch process, but at a certain time of feeding substrate is added into the culture. In cultivation of *Scenedesmus*, removal of half of culture and addition a new culture media into the culture is done. In this study, growth parameters such as light, pH, temperature and agitation of culture are controlled. Light is important source of energy as microalgae utilize this source to photosynthesize assimilation of conversion of inorganic carbon to organic matter (Encarnación *et al.*, 2010). Light intensity can influence growth rate since increase light density of culture have certain limit. High light intensity also can caused growth was inhibited (Mata *et al.*, 2010). Lighting for *Scenedesmus* cultivation in the fermentor is controlled 12 hours on and 12 hours off. The pH is controlled at pH 9.0 which is optimum growth temperature for *Scenedesmus*. Optimum growth temperature of *Scenedesmus* at 30-35°C as stated by Encarnación et al. (2010). Thus, temperature in the B-Braun fermentor for cultivation of *Scenedesmus* is controlled at 30°C. *Scenedesmus dimorphus* is a heavy bacterium which is formed thick sediment if not kept in constantly agitation. Thus, agitation is essential as it can facilitate nutrient distribution in the fermentor. According to Mata *et al.* (2010), mixing is essential growth parameter because

it homogenizes the cells distribution, heat, metabolites and facilitates transfer of gases. However, high speed agitation or mixing may damage the cells due to shear stress. Thus, in this study agitation constantly controlled at 200 rpm which is suitable for cultivation of *Scenedesmus* and does not damage the cells.

3.0 MATERIALS AND METHODS

The experiments are conducted according to flow chart in **Figure 3.1**.

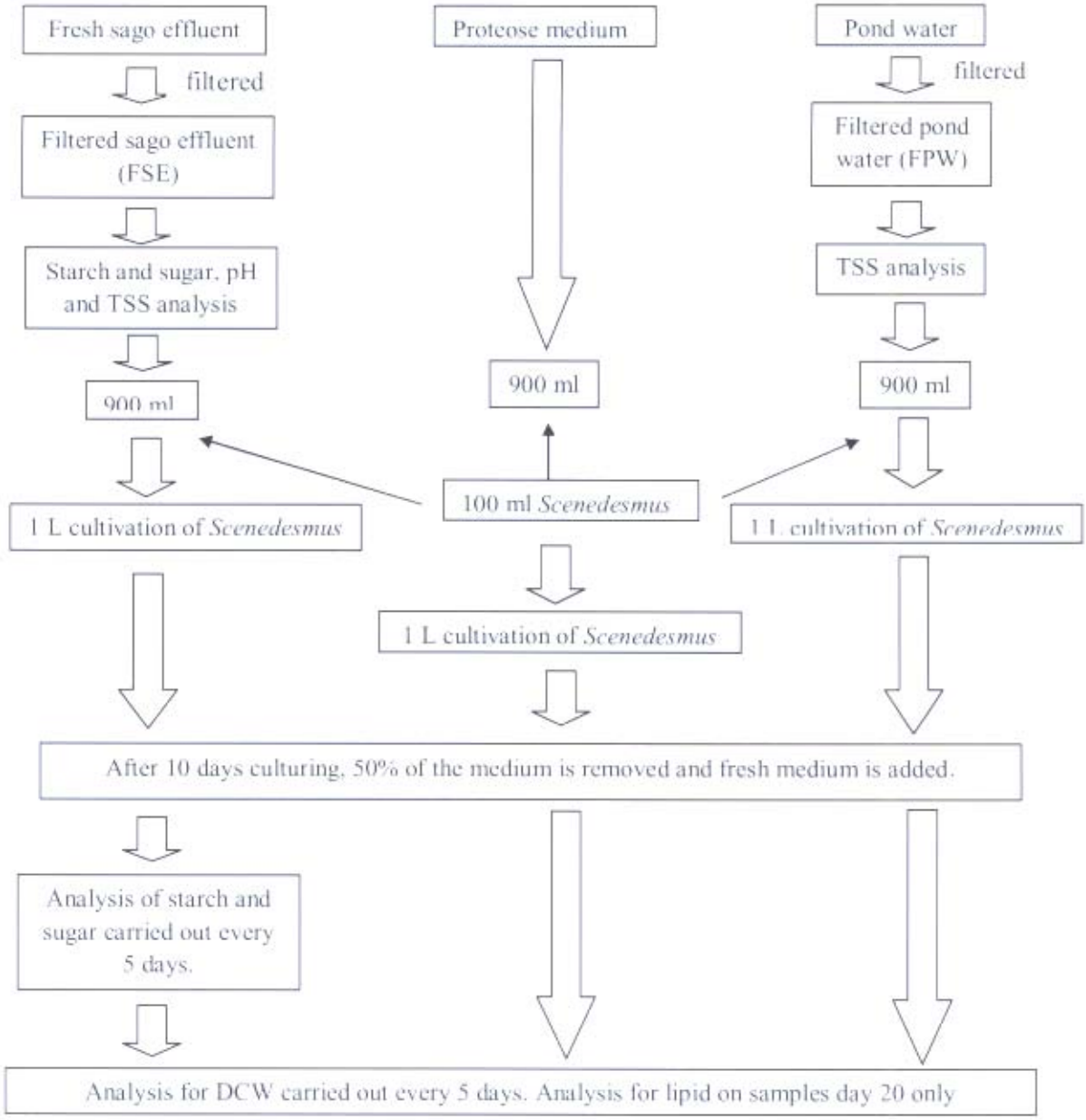

Figure 3.1: Flow chart of Material and Methods.

3.1 MATERIALS

3.1.1 Microorganism

Scenedesmus dimorphus strain was obtained from University of Texas, USA and was used in this study. 10% v/v of *Scenedesmus* was used in all culture media. Stock of *Scenedesmus* was sub-cultured to maintain sufficient supply. Culture at optical density more than 0.5 but less than 1.0 were harvested and used as inoculums. The stock of *Scenedesmus* was cultured in 1 L Erlenmeyer flask and bubbled with air (**Figure 3.2**). The optical density (OD) of the cultures was checked first before use for culture in filtered sago effluent or filtered pond water or Proteose medium.

Figure 3.2 Stock Culture of *Scenedesmus dimorphus*